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Length-weight relationship and reproductive activity of the *Leporinus piau* Fowler, 1941 captured in a small deactivated hydropower plant

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ABSTRACT. Reproductive activity of 'piau gordura' *Leporinus piau* was studied using biometric, macroscopic and microscopic analysis. A total of 74 specimens of *L. piau* were collected quarterly from January to December 2005 in a small deactivated hydropower plant located at Jorge Pequeno Stream and preserved in a fixative solution until analysis. The alterations after fixation were evaluated. The stages of gonadal maturation were determined by histology and gonadosomatic index (GSI). The females and males in the spawning capable and regressing stage were registered from October to December. *L. piau* is total spawning and the HSI (hepatossomatic index), SRI (stomach repletion index) and CFI (coelomic fat index) of females and males did not showed a statistical difference. However they were numerically different between the stages of maturation. For both sexes, the smallest specimens captured during the reproductive activity have measured around 8.3 cm in total standard. The length-weight relationship observed for the parameter 'b' was 3.01 and the parameter 'a' was 0.02. This study demonstrates the importance of Jorge Pequeno Stream in the reproductive activity of migratory fish in the upper São Francisco river.

Keywords: Anostomidae, reproduction, biological indexes, tributary, São Francisco river.

Relação peso-comprimento e atividade reprodutiva do *Leporinus piau* Fowler, 1941, capturados em uma pequena usina hidrelétrica desativada

RESUMO. A atividade reprodutiva do 'piau-gordura' *Leporinus piau* foi estudada, utilizando análises biométricas, macroscópicas e microscópicas. Um total de 74 exemplares de *L. piau* foram capturados trimestralmente de janeiro a dezembro de 2005 em uma pequena usina hidrelétrica desativada localizada no Ribeirão Jorge Pequeno e fixados até as análises biométricas e histológicas. As alterações após fixação foram avaliadas. Os estádios de maturação gonadal foram determinados por histologia e índice gonadossomático (IGS). As fêmeas e os machos capazes de desovar foram registrados de outubro a dezembro. *L. piau* possui desova total e os índices IHS (índice hepatossomático), IRS (índice de repleção estomacal) e IGC (índice de gordura celômica) de fêmeas e machos não apresentaram diferença estatística apesar de numericamente serem diferentes entre os estádios de maturação. Os menores exemplares capturados em atividade reprodutiva mediram 8,3 cm de comprimento total para ambos os sexos. Na análise da relação peso-comprimento, o parâmetro 'b' foi 3,01 e o parâmetro 'a' foi de 0,02. Este estudo fornece importantes informações sobre *L. piau* e sugere o papel do Ribeirão Jorge Pequeno na atividade reprodutiva de peixes na região do alto rio São Francisco.

Palavras-chave: Anostomidae, reprodução, índices biológicos, tributários, rio São Francisco.

Introduction

Reproductive behavior varies greatly in teleost fishes depending on the environmental conditions but also on reproductive strategies. In iteroparous species, spawning pattern will depend on the rhythm of ovulation: (a) total spawning which the oocytes are released during a short period of time, and (b) multiple spawning where oocytes are released in several batches throughout the spawning season (JALABERT, 2005; LUBZENS et al., 2010). Knowledge on the spawning strategy is gaining importance in restoration efforts in large rivers to benefit endangered species (BREWER et al., 2008).

The variation of biological indexes during the reproductive cycle represents the way the species uses the energetic resource from the environment, defining its life history strategies (VAZZOLER, 1996). Life history parameters such as sex ratio may vary between populations of a species and temporally within population (MORGAN, 2008). In fisheries biology, length-weight relationships are useful in determining weight and biomass when only length measurements

are available, as indications of condition and to allow for comparisons of species growth between different regions (KOUTRAKIS; TSIKLIRAS 2003; FROESE, 2006). Many fields in fish biology needs on morphological data that are often obtained from individuals that must be fixed until the measurement, and the effects of preservation on fish morphometrics must be considered (SAGNES, 1997).

The São Francisco river headwaters are in the Canastra Hills in southwestern Minas Gerais, extends 2.700 km and empties into the Atlantic Ocean. The river is heavily regulated, its waters are used mainly for power generation, irrigation, urban supply and industrial (SATO; GODINHO, 2003). The upper São Francisco river basin is formed by small water courses, and these tributaries enable the propagation of numerous species of fish. Furthermore, several marginal lagoons used as recruiting grounds for fishes were destroyed for agricultural production (SATO; SAMPAIO, 2006).

The piau gordura, *Leporinus piau*, is a middle-sized species, an omnivorous forage fish, abundant along the entire São Francisco river basin, including tributaries with commercial value in fisheries (SAMPAIO; SATO, 2009). Displays non-adhesive eggs with fibrillar networks (RIZZO et al., 2002), and feeding mainly on invertebrates and fruits and is also registered filamentous algae, grass roots, seeds and zoobenthos (NAKATANI et al., 2001; MONTENEGRO et al., 2010). Currently, no information exists concerning reproductive parameters of fishes in neotropical environments from a small deactivated hydroelectric power plant. The major goal of this work was to describe the reproductive activity and to investigate the length-weight relationship after fixation of *L. piau*, in the upper São Francisco Basin, south-eastern Brazil.

Material and methods

Fish were captured in a reservoir with a drainage area of 84 km^2. A small deactivated hydropower plant, located in Jorge Pequeno stream (19° 49'S, 45° 40'W), which is considered a first order tributary of the upper São Francisco river in south-eastern Brazil, Luz city, Minas Gerais State. The distance between the dam and the upstream of Jorge Pequeno stream into São Francisco river is 29.6 km. The right side of the reservoir is characterized by pasture area and the left side showed preserved riparian forest.

A total of 74 specimens of *L. piau* were collected quarterly from January to December 2005. Fish were caught using gillnets with mesh size ranged from 1.5 to 6.0 cm (stretched mesh). These nets had 10 m long with 2.0 m height. Two nets were utilized by sample with soaking time approximately 14h. Fish were killed by transverse sectioning of the spinal cord following the ethical principles established by the Brazilian College of Animal Experimentation (COBEA). Specimens were fixed in 10% formaldehyde solution and after dissection the following parameters were recorded: standard length (SL), body weight (BW), sex, gonad weight (GW), liver weight (LW), stomach weight (SW) and coelomic fat weight (CFW). Biometric data were used to determine the following biological indexes: gonadosomatic index (GSI = 100GW BW^{-1}), hepatosomatic index (HSI = 100LW BW^{-1}), stomach repletion index (SRI = 100SW BW^{-1}) and coelomic fat index (CFI = 100CFW BW^{-1}).

Five specimens of 'piau gordura' were caught with casting net to investigate the effect on measurements length and weight after fixation in 10% formaldehyde solution. Each individual was anesthetized with tricaine methanosulfonate (Sigma) and, then biometric data were obtained on fresh fish. After the fixation, fish were measured and weighed each three days during 21 days. The analysis of length and weight changes after fixation was performed according the equation: Alteration = preserved value – fresh value/fresh value x 100 (SAGNES, 1997). The relationship between the initial (Y) and preserved (X) measurements was calculated using the linear regression Y = 'a + b'.X, where 'a' is the intercept and 'b' the slope (PARADIS et al., 2007). The degree of association between the variables Y and X was evaluated by the correlation coefficient (R^2). For analysis of gonadal maturity phase, samples of the middle region were obtained from the gonads of all specimens caught. These fragments were fixed in Boiun's fluid during 8h at room temperature, embedded in paraffin, sectioned at 5 μm thickness and stained with hematoxylin-eosin (HE). Gonadal maturation was evaluated on the basis of macro- and microscopic characteristics of the gonads and on the variation of GSI. The gonadal classification was adapted from Brown-Peterson et al. (2011).

Mean values of the biologic indexes calculated by sex for each gonadal maturation stage were compared using one way ANOVA followed by Tukey´s *post hoc* test to determine significant differences between mean values. The reproductive period was determined by taking into account the frequency distribution of the different gonadal maturation. A Chi-square ($\chi 2$) test was used to assess the significance of differences in the sex ratio of males to females. Additionally, the length-weight relationship was calculated using the equation $W = aL^b$, where 'a' is a coefficient related to body form and 'b' is an exponent indicating isometric growth when equal to 3. The parameters 'a' and 'b' of the exponential curve were estimated by linear regression analysis on the log-transformed data. The degree of association between the variables W and L was evaluated by the correlation coefficient (R^2).

Results

Ovaries and testes are paired, elongated and fusiform organs, located inside the coelomic cavity and attached to the air bladder by the mesovarium and the mesorchium, respectively. By light microscopy, gonads appeared coated by the tunica albuginea, which emitted septae to the interior of the ovaries forming ovigerous lamellae with oocytes, or lobes with seminiferous tubules containing spermatogenic lineage cells in testes.

Gonadal activity showed seasonal changes, thus allowing the characterization of three main phase according the macro and microscopic characteristics of ovary and testis: 1 - regenerating, 2 - spawning capable and 3 - regressing. In the regenerating ovaries, the ovigerous lamellae showed oogonia nests and perinucleolar follicles, with oocytes presenting basophilic cytoplasm, a central vesiculous nucleus and several peripheral nucleoli (Figure 1A). In males the seminiferous tubules showed the lumen closed with many spermatogonial cells (Figure 1D). Spawning capable phase was characterized in females by the presence of vitellogenic follicles filled of yolk globules (Figure 1B), and in males the seminiferous tubules full of spermatozoa (Figure 1E). After spawning, regressing phase, postovulatory follicles and atretic follicles were observed in the ovaries (Figure 1C) and the lumens of the seminiferous tubules were empty (Figure 1F).

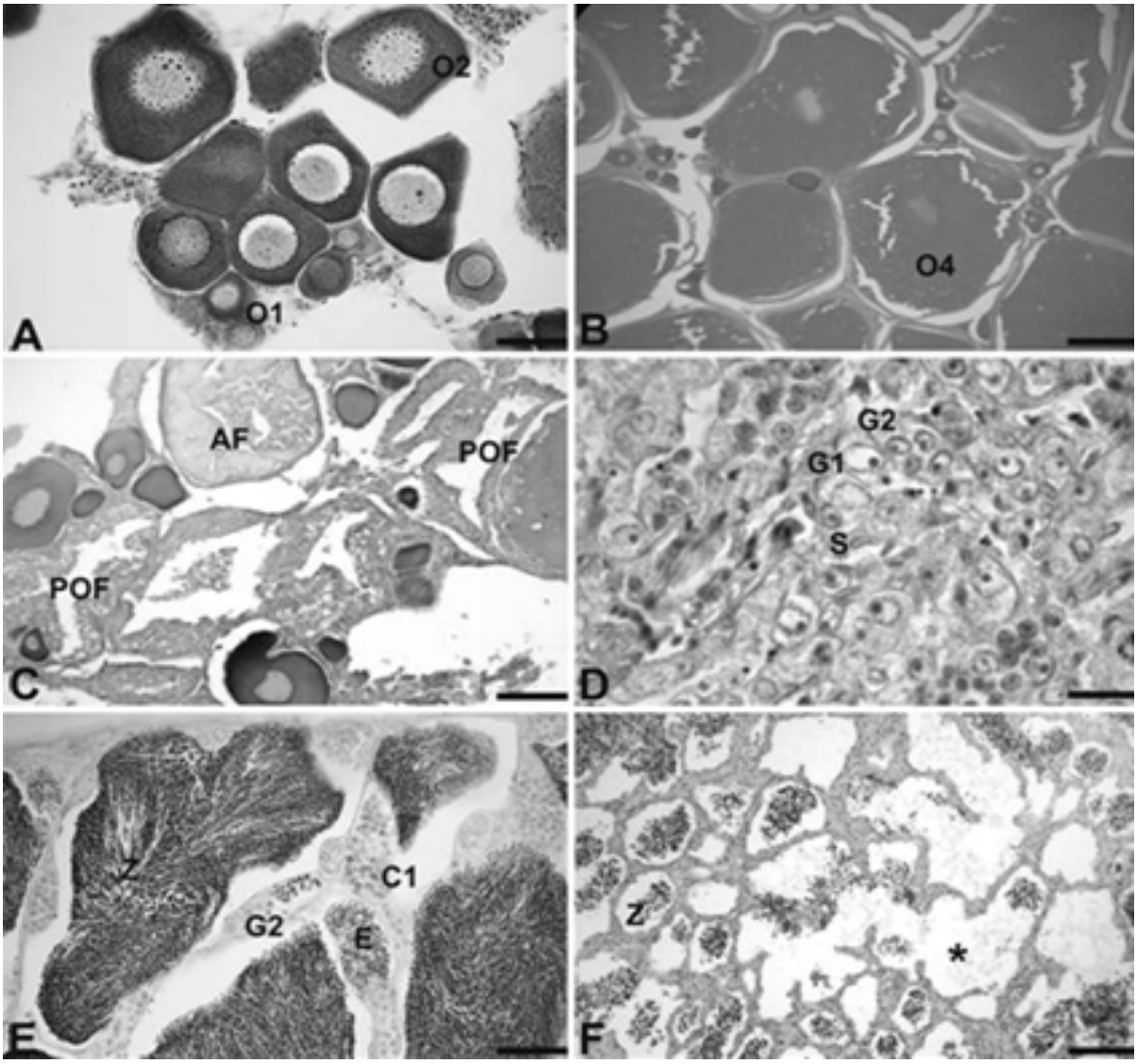

Figure 1. Microscopic characteristics of the different stages of gonadal maturation stages in females (A-C) and males (D-F) of *L. piau* captured in Jorge Pequeno Stream, Minas Gerais, HE staining. A – Regenerating: Ovaries containing only initial (O1) and advanced (O2) perinucleolar oocytes; B – Spawning capable: ovaries with predominance of vitellogenic oocytes (O4); C – Regressing: with post-ovulatory follicles (POF) and atretic follicles (AF) that remain after the spawn; D – Regenerating: seminiferous tubules with closed lumen, spermatogonia (G1 and G2) and Sertoli cell (S); E – Spawning capable: seminiferous tubules with cysts of spermatocytes (C1), spermatids (E) and lumen full of spermatozoa (Z); F – Regressing: emptied seminiferous tubules with opened lumen (*), presenting some residual spermatozoa (Z). Bars = 41 μm (A); 60 μm (B); 40 μm (C); 35 μm (D); 18 μm (E); 20 μm (F).

Regenerating females occurred during January to March while the peak of spawning capable stage occurred between October and December for both sexes. The peak of spawned females was registered from October until December (Table 1). Short spawning period, absence of partially spawned females indicated that *L. piau* presented total spawning.

Table 1. Frequency of the gonadal maturation stages of *L. piau* females and males captured in Jorge Pequeno stream, tributary of São Francisco river, from January to December, 2005.

Period/Year	Females			Males			Total
	1	2	3	1	2	3	
Jan-Feb-Mar	12	1	1	5	10	6	35
Apr-May-Jun	3	-	1	1	2	2	9
Jul-Aug-Sep	-	-	1	-	-	-	1
Oct-Nov-Dec	1	6	11	-	11	-	29
Total	16	7	14	6	23	8	74

Stages: 1) regenerating, 2) spawning capable, 3) regressing.

Mean GSI values calculated for males and females accompanied the maturation of the gonads. Spawning capable males and females showed significant differences of GSI among others phases. The HSI in males varied discretely along the reproductive cycle. In females, the lowest HSI and CFI values were registered during the spawning capable phase. The highest SRI in females was observed during the regressing phase. For males, this index and the CFI varied discretely between the reproductive phases. In both males and females, there were no significant differences found between the phases (Figure 2).

During the study period, 37 males (50%) and 37 females (50%) of *L. piau* were captured, indicating a sex ratio of 1:1, showing no significant differences ($\chi^2 = 0.0$, df = 1, $p > 0.05$). The largest specimen captured was a male of 19.7 cm (SL) and 192.0 g (BW). The mean SL and BW of Spawning capable females were 10.74 ± 1.49 cm and 41.00 ± 20.78 g, respectively. Among the Spawning capable males, the mean SL and BW were 11.17 ± 2.67 cm and 48.00 ± 37.88 g, respectively. The smallest female and male found in reproductive activity measured 8.3 cm in both sexes (Table 2).

Table 2. Values of standard length and body weight grouped by gonadal maturation stages (GMS) of *L. piau* females and males captured in Jorge Pequeno stream, tributary of São Francisco river, from January to December 2005.

Sex	Standard Lenght			Body Weight	
	GMS	Mean ± SD	Range	Mean ± SD	Range
Females	1	9.98 ± 0.94	8.6 – 11.7	28.00 ± 0.94	16 – 42
	2	10.74 ± 1.49	9.0 – 13.0	41.00 ± 20.78	22 - 75
	3	13.06 ± 3.39	8.3 – 18.0	76.00 ± 52.88	16 – 168
Males	1	8.55 ± 0.21	8.8 – 8.3	18.00 ± 1.98	15 – 21
	2	11.17 ± 2.67	8.3 – 17.1	48.00 ± 37.88	15 – 149
	3	11.50 ± 3.86	8.3 – 19.7	32.00 ± 18.18	18 - 192

Stages: 1) regenerating, 2) spawning capable, 3) regressing.

The length-weight relationship was estimated for *L. piau*: parameter 'b' was 3.01 and parameter 'a' was 0.02 with correlation coefficient (R^2) equal to 0.97. The equations corresponding to length-weight relationship was done for the males (Figure 3A) and females (Figure 3B), separately.

There was no statistical difference between the percentage alterations in the biometric data of weight and length after fixation in the times studied. The most marked changes correspond to the length, while the weight tends to decrease the difference between fresh weight and preserved weight. The relationship between the initial and preserved measurements to length revealed that the slopes and intercept of the regressions stabilized in 12 days after the fixation, with the correlation coefficient equal to 0.94. All the regression equations were highly significant, R^2 values ranging from 0.94 to 0.99 (Table 3).

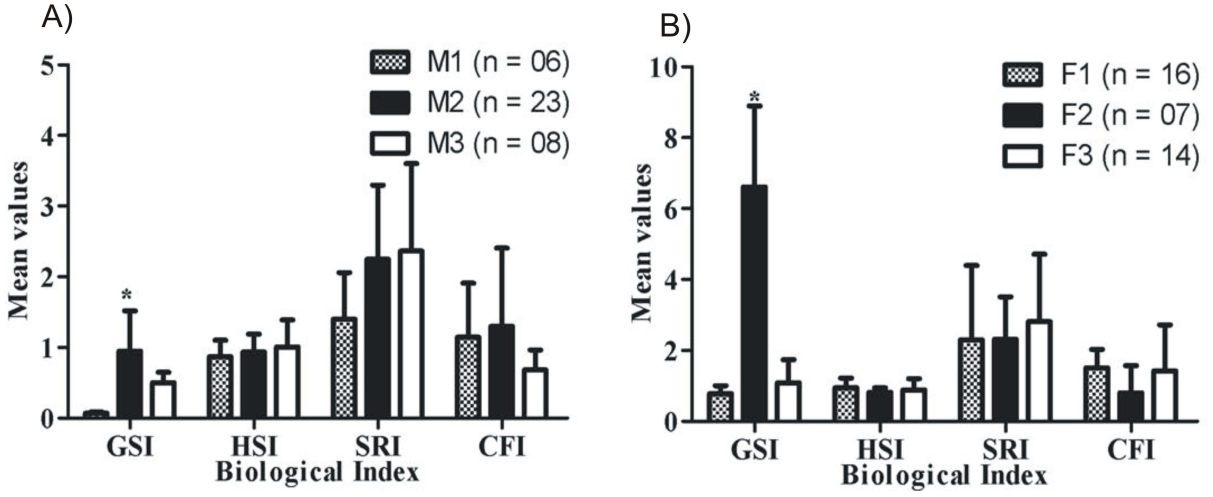

Figure 2. Mean values ± SD of the gonadosomatic (GSI), hepatosomatic (HSI), stomach repletion (SRI) and coelomic fat (CFI) indexes according to gonadal maturation stages of males (A) and females (B) of *L. piau* sampled in Jorge Pequeno stream, from January to December 2005 [Regenerating (dotted), spawning capable (filled column), regressing (blank column)]. Values followed by asterisk above the bars of each index are significantly different (one-way ANOVA, $p < 0.05$).

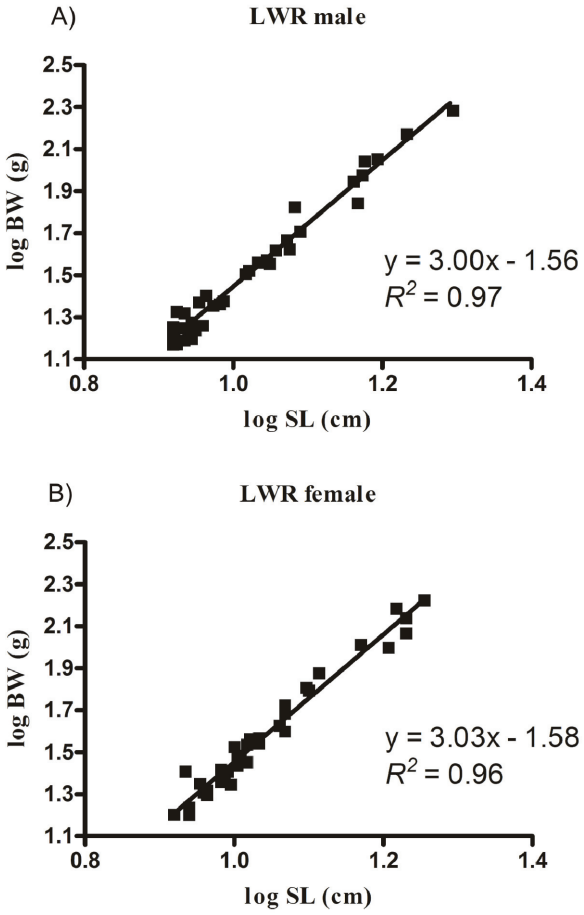

Figure 3. Length-weight relationship of *L. piau* males (A) and females (B) sampled in Stream Jorge Pequeno, tributary of São Francisco river, from January to December 2005.

Table 3. Parameters ('a' and 'b') of linear regression equations used to convert preserved lengths (cm) and weights (g) of *L. piau* to fresh values.

Preservation period	3	6	9	12	15	18	21
			Length				
Parameter *a*	1.07	1.06	0.90	1.13	1.13	1.13	1.13
Parameter *b*	0.93	0.93	0.93	0.92	0.92	0.92	0.92
R^2	0.94	0.94	0.94	0.94	0.94	0.94	0.94
			Weight				
Parameter *a*	12.13	12.14	12.08	12.97	10.75	10.71	10.60
Parameter *b*	0.97	0.97	0.97	0.96	0.96	0.96	0.96
R^2	0.99	0.99	0.99	0.99	0.99	0.99	0.99

All regression equations were significant ($p > 0.05$).

Discussion

Despite the extensive river system represented by the streams in Brazilian river basins, there are few studies involving fish reproduction. In Jorge Pequeno stream, which is part of São Francisco river basin, there are no studies on neotropical ichthyofauna. This study reported the length-weight relationship and reproductive activity of *L. piau* in upper São Francisco basin and that species use of Jorge Pequeno stream as a spawning site. Tributaries may act as alternative routes to several species of fish, playing an important role in the maintenance of regional biological diversity and of fish stocks (SATO et al., 2003; GUBIANI et al., 2010).

Ovarian and testicular morphology varies among teleosts are important for phylogenetic analysis and for the understanding of gonadal evolution (PARENTI; GRIER, 2004; LUBZENS et al., 2010; MARTINS et al., 2010). The morphology of the gonads in *L. piau* is similar to other Brazilian anostomids already described (TAVARES; GODINHO, 1994; RIZZO et al., 1996; RICARDO et al., 1997; BRITO et al., 1999; LOPES et al., 2000; COSTA et al., 2005; THOMÉ et al., 2005).

In the present study, the ovaries were identified in cystovarian type, and showed the ovigerous lamellae with oogonia and ovarian follicles, which corresponds to the same organization found in most teleosts (GRIER et al., 2009). The seminiferous tubules of *L. piau* intercommunicated with one another forming an anastomosed network, from the periphery to the testicular duct region, classified as 'anastomosing tubular testis' as already related in the literature on basal fishes (GRIER; URIBE-ARANZÁBAL, 2009).

The reproduction of teleosts depends on environmental factors that vary in each region, resulting in different forming and scales for reproductive species is different habitats (WOOTON, 1998). In the literature, the number of gonadal maturation stages registered varies depending on the criteria used to define them, the characteristics of the gonads and sample size (BAZZOLI, 2003; BROWN-PETERSON et al., 2011; NUÑEZ; DUPONCHELLE, 2009). The frequency analysis of maturation phases of L. piau showed fish in reproductive activity occurred mainly between October and March, featuring the seasonality of the reproductive period, with peaks in October, November and December, coinciding with the reproduction season of other Brazilian Anostomidae, such as *Leporinus reinhardti* (RIZZO et al., 1996), *Leporinus striatus* (RICARDO et al., 1997), *Leporinus friderici* (BRITO et al., 1999) and *Leporinus taeniatus* (THOMÉ et al., 2005).

The *L. piau* captured in Jorge Pequeno stream was characterized by histological analysis and reproductive period as total spawning type. Periods of high temperatures and rain intensity contribute to the processes that induce maturation and spawning of fish (BRAGA, 2001). In fish ovaries, total spawners have a single mature oocyte size, whereas in multiple spawners the fully matured oocytes are found among others in different development stages (NUÑEZ; DUPONCHELLE, 2009).

The gonadosomatic index (GSI) with the histological analysis of the gonads, is commonly used to determine the annual reproductive cycle of fish (BREWER et al., 2008). Increasing GSI values

are associated with maturation and low GSI values with eliminating or resorption of gametes (BAZZOLI, 2003). We observed a gradual increase of GSI values in both sexes, in stages regenerating to the spawning capable, and its reduction in phase regressing, as typical for neotropical teleosts (VAZZOLER; MENEZES, 1992). In the female HSI and CFI were lower in Spawning capable stage. Lipids, the main source of energy reserves of fish, are accumulated in the liver, muscle and adipose tissue and are mobilized to supply the energy demand as growth, maintenance and reproduction (MOREIRA et al., 2000). Moreover, these indexes are used as indicators of the reproductive period and related to the relocation of energy resources from the liver to the vitellogenesis process (TAVARES-DIAS et al., 2000; QUEROL et al., 2002). The SRI values were greater during the post-spawning, indicating that females increased food consumption to replace the energy lost due to reproduction according to other teleost species (BAZZOLI et al., 1998; MADDOCK; BURTON, 1999; RATTON et al., 2003). In males, the values of HIS, SRI and CFI ranged slightly. This fact may be related to mobilization and accumulation of energy reserves, which can be used in the reproductive or non reproductive (MADDOCK; BURTON, 1999; QUEROL et al., 2002).

Sex ratio provides subsidies for the study of population dynamics in fish, and usually for neotropical fishes it is 1:1, and may vary from species to species within the same population and from one year to another (NIKOLSKY, 1963). In the Jorge Pequeno stream, *L. piau* showed the population structure of 1:1 females to males, while for *L. taeniatus* collect at the Juramento reservoir in Minas Gerais the sexual proportion 1:1.3 females to males was observed (THOMÉ et al., 2005).

The smallest body size at which a species begins its reproductive activity is called the size of sexual maturity (BAZZOLI, 2003). In the present study, the smallest specimen on this condition of females and males was 8.3 cm. Our data corroborates the size of first maturation established for *L. piau* at Três Marias reservoir (TAVARES; GODINHO, 1994; SOARES et al., 1996). The first gonadal maturation size is a parameter that provides subsidies to the fisheries regulations (BAZZOLI, 2003).

In the present study, the length-weight relationship was established for *L. piau* and separately to males and females. The parameter 'b' was equal 3 independently of sex, indicating that the small specimens sampled have the same form and condition as large specimens (FROESE, 2006). On the other hand, for *L. piau* captured in Taperoá II Dam the parameter 'b' observed was smaller than 3 (MONTENEGRO et al., 2010), indicating that large specimens have changed their body shape to become more elongated or small specimens were in better nutritional condition at the time of sampling (FROESE, 2006). The length-weight relationship in fishes is affected by different factors including diet, stomach fullness, health, and preservation techniques (RICKER, 1968).

The effects of fixatives on fish morphometric evaluation are difficult to predict because of variance related to type of fixative, duration of preservation, species and life stage (SAGNES, 1997). The assessment of formaldehyde solution effect on the morphometric analysis for *L. piau* indicated that the length measurement is the most affected. However, this measure did not change after 12 days of fixation. The linear regression equation for the length showed that the parameters 'b' and 'a' were stable after 12 days. Thus this equation could be used to convert the preserved measurements of the length in fresh values acting as a correction factor, with a high coefficient of correlation, in *L. piau* and others Anostomids.

Conclusion

Despite the many factors impacting the Jorge Pequeno stream, the development condition for small and large specimens of *L. piau* is the same. In addition, reproductive activity of this species was recorded, suggesting that this stream play an important role in maintaining fish stocks in the upper São Francisco.

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